NTP
National Toxicology Program

Toxicology Studies of Evening Primrose Oil in Harlan SD Rats and B6C3F1 Mice

Dori R. Germolec, Ph.D.
National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors
December 10, 2009

Nomination Background and Rationale

- Evening Primrose Oil (EPO), is a biennial weed that is a minor oilseed crop used to produce the dietary supplement
  - All the plant parts are edible
  - Source of essential fatty acids, particularly linoleic acid (LA) and γ-linolenic acid (GLA)
- Nominated by the NIEHS for toxicological characterization based on:
  - Widespread use in dietary supplements
  - Lack of adequate toxicological data
  - Concern regarding potential adverse effects

Human Exposure to EPO

- Human exposure primarily through the consumption of EPO containing dietary supplements
- Routinely in the Top 20 in Herbal Sales
- Licensed for the treatment of mastalgia, PMS and prostatitis in the UK
- Suggested use for treatment of a variety of inflammatory conditions:
  - PMS, atopic eczema, psoriasis, multiple sclerosis, cancer, coronary heart disease, diabetic neuropathy, autoimmune conditions, and gastrointestinal symptoms
- Also used during pregnancy for prevention of pre-eclampsia and early delivery, and shortening and stimulating labor

EPO - Human Studies

- Most human studies have examined efficacy for anti carcinogenic effects, treatment of neurologic disorders and modulation of labor and delivery
  - EPO is well tolerated by most people, but long-term safety has not been evaluated in human studies
  - Mild side effects include gastrointestinal upset and headache
  - Some cancer patients have objective improvement following EPO treatment, but results are variable
  - Use of EPO was associated with increases in labor time and active phase labor abnormalities
    - incidence of prolonged rupture of membranes, protracted active phase, oxytocin augmentation, arrest of descent, cesarean delivery, and vacuum extraction.
  - Early suggestions of association with seizures not supported by review of the studies
    - Precautions for use suggest not to take if you have psychiatric disorders because of potential interactions with phenothiazines

EPO Toxicity Studies - Long term studies

- No significant differences in body weight, weight gain and food consumption in mice, rats and dogs fed EPO
- Minimal effects in a chronic toxicity study in SD rats and beagle dogs
  - Rats - 0.3, 1 and 2.5 mL/kg EPO by gavage for up to 53 weeks
    - Increased potassium level in female rats at the 2.5 mL/kg dose
    - Reduced liver weights in males but no histopathology
  - Dogs - 1, 3 and 5 mL/kg EPO by gavage for up to 52 weeks
    - No differences in gross lesions or histopathology, clinical chemistry or hematology
  - (Everett et al., 1988)

EPO Toxicity Studies - Carcinogenicity studies

- No significant differences in tumor incidence, but a trend for fewer tumors in male rats and all mice
  - SD Rats - 0.3, 1 and 2.5 mL/kg EPO by gavage for up to 104 weeks
  - CD-1 mice - 0.3, 1 and 2.5 mL/kg EPO by gavage for up to 78 weeks
  - No list of tissues given in this paper, but in the chronic toxicity paper the list of organs and tissues take is very complete
  - (Everett et al., 1988)

EPO Effects on Pregnancy and Reproduction

- Use of EPO was associated with labor abnormalities in nulliparous women
  - Increased labor time and incidence of prolonged rupture of membranes, protracted active phase, oxytocin augmentation, arrest of descent, cesarean delivery, and vacuum extraction.
- Rodent studies
  - SD Rats - 0.3, 1 and 2.5 mL/kg EPO by gavage for up to 53 weeks
    - Six out of 25 males receiving 2.5 mL/kg (and two of 25 controls) demonstrated testicular shrinkage or softening (Everett et al., 1988)
  - Wistar Rats fed EPO as 3% of total dietary fatty acids
    - No differences in parturition, birth weight, postnatal growth rate, maternal weight during pregnancy, and fetal or placenta prostaglandin E2 levels as compared to control animals
  - Male ICR mice - 0.5 mL EPO daily for 28 days
    - Body weight, testis weight, testosterone levels and the number of sperm positive females and complete penile insertions during a three-hour period were increased

EPO - Genotoxicity and Immunology Studies

- Genotoxicity
  - No evidence that EPO is genotoxic
  - Reduced incidence of micronuclei when co-administered with Benzo(a)pyrene suggest that it may prevent DNA adducts
- Immunotoxicity
  - Isolated case reports of hypersensitivity following exposure to EPO
  - Some evidence of immunosuppression in rodents
    - Reduction in natural killer cell activity in Lewis rats
    - Reduced secretion of pro-inflammatory mediators
    - Protective effect for some autoimmune diseases in rodents and humans but enhancement of disease manifestations in others

EPO - Toxicokinetics

- The metabolism of essential fatty acids is well understood in humans and laboratory animals
- Animal tissues are more active in conversion of LA to longer-chain polyunsaturated fatty acids (PUFAs) than humans
- There is limited data on the bioavailability of LA and GLA following EPO treatment

Proposed Research Program - Key Issues

- As with other dietary supplements, a key question is what product or formulation to study. Commercial preparations vary in their fatty acid content and many of these are also supplemented with other PUFAs (such as fish oil) and/or antioxidants.
- The available toxicology data is extremely limited, but there is little evidence of systemic toxicity following EPO consumption in humans or experimental animals.
- The pattern of use and reported effects on reproductive endpoints suggest that additional studies in this area would be warranted
- Evidence of immune effects, but most consistent with what has been reported in the literature with regard to essential fatty acid supplementation
  - Isolated reports of hypersensitivity, but no rodent studies

Proposed Research Program (continued)

- The overall goal of these studies is to characterize the subchronic and reproductive toxicity of EPO following oral exposure in rats and mice. The following tiered approach with defined specific aims will address this goal:
  - Tier One
    - Specific Aim 1 - Conduct studies on the bioavailability of EPO
    - Specific Aim 2 - Conduct prechronic toxicity studies in the Harlan SD rat and B6C3F1 mouse
      - Rat studies will be conducted using the perinatal exposure paradigm
      - These studies will address the lack of toxicity data for EPO and provide information on the potential targets of EPO toxicity
      - These studies will provide critical information for dose setting in the RACB

Proposed Research Program (continued)

- Tier Two
  - Specific Aim 3 - Conduct a guideline reproductive toxicity study to examine the effects of EPO on fertility and fecundity in SD rats
  - Specific Aim 4 - Conduct a standard immunotoxicity screening panel to assess the potential immunomodulatory effects of EPO
- Tier Three
  - Evaluate the need for additional toxicity studies based on the results of Specific Aims 1 and 2

Significance and Expected Outcomes

- Information on the number of individuals who take EPO is not readily available
- The reported effects on pregnancy and reproductive endpoints may be a cause for concern. These studies will address data gaps in this area
- Additional studies will address the lack of toxicity data for EPO and provide much-needed information on its safety for the FDA and the public